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Metal Carbonyls for Site-specific FTIR Microspectroscopic Localization

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Introduction: FTIR microspectroscopy has been used to demonstrate differences between diseased and healthy tissue, changes in the chemical composition that are dependent on the state of differentiation, and metabolic differences between cellular layers [1]. Recent developments in infrared array detectors, viz., focal plane array detectors (FPA), have led to the collection of spectroscopic images with unprecedented fidelity [2]. Intense infrared absorption bands of metal carbonyls in the region, 2200 to 1850 cm^{-1}, have led to their use as non-isotopic organometallic markers for biological assays [3,4]. We wish to develop functionalized metal carbonyls as site-specific probes for FTIR-microspectroscopy.

Methods and Materials: metal carbonyls, cellulose acetate, nylon and Teflon membranes.

Results: Previously, we found that the derivatized metal carbonyls had low solubilities in the aqueous buffers used in biological assays. Therefore, hydroxy- substituted amine functionalized phosphines were synthesized for better compatibility with aqueous buffers. Mono-phosphine substituted tri-osmium, and tri- and tetra-phosphine substituted tetra-iridium carbonyl clusters were synthesized using new phosphine derivatives. As low as 200 nM of the iridium and 125 nM of the osmium clusters, spotted on PTFE IR disposable cards could be detected in transmission mode. Thickness of the blotting membranes and their non-reflective nature prevented sensitive detection of metal carbonyls in transmittance and ATR mode on cellulose acetate and nylon membranes. In ATR mode, diamond probe also reduced the sensitive detection due to inherent diamond absorption bands in the region of interest.

Immunoblots, therefore, were carried out on PTFE membranes. However, permanent immobilization of target antibody on PTFE proved to be difficult. Approximately 75% of the target antibody washed off from the membrane during the assay. Secondly, up to 50% immunospecificity of the target protein was also compromised. The loss of immunoreactivity and target protein itself drastically reduced the sensitivity of detection of the dot-blot assays. Metal carbonyls could be detected with better sensitivity (~75 nM) in refection mode when spotted on IR reflective slides. The lower sensitivity in transmission mode could be attributed to the thickness of the PTFE membrane. The target antibody could not be permanently immobilized on IR reflective slides also.

Conclusions: Permanent surface modification of PTFE membranes and IR reflective coated glass slides for immobilization of the target protein is necessary for improved sensitivity of detection.

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References:

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